



Atty Dkt. No.: CLON-056CIP
USSN: 09/858,332

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DECLARATION UNDER 37 C.F.R. §1.131 Address to: Commissioner for Patents P.O. Box 1450 Alexandria VA 22313-1450	Attorney Docket	CLON-056CIP
	Confirmation No.	2176
	First Named Inventor	Tchaga, Grigoriy S.
	Application Number	09/858,332
	Filing Date	May 15, 2001
	Group Art Unit	1652
	Examiner Name	Kathleen M. Kerr
	Title	<i>POLYNUCLEOTIDES ENCODING METAL ION AFFINITY PEPTIDES AND RELATED PRODUCTS</i>

Sir:

This Declaration and the attached Exhibit are being submitted in conjunction with the Applicants' Response to the Office Action dated May 6, 2005.

We, Grigoriy S. Tchaga, and George G. Jokhadze, do hereby declare as follows:

1. We are the listed co-inventors of the above-captioned application.
2. Enclosed with this declaration is Exhibit A that provides evidence of conception and reduction to practice of the claimed invention prior to July 1998.
3. Specifically, Exhibit A is a series of laboratory notebook pages, all of which were in existence prior to July 1998), that describe incorporation of a polyhistidine metal ion affinity sequences at the terminal sequence of a recombinant DHFR protein and use of the affinity sequence for the purification of the recombinant DHFR protein with high selectivity.

- a. Page 1 of Exhibit A briefly describes the purification protocol for the HAT-DHFR using FPLC under native conditions and also by batch-gravity flow under denaturing conditions.
 - b. Page 2 of Exhibit A shows preliminary purification results for the HAT-DHFR.
 - c. Page 3 of Exhibit A is a letter from ATG Laboratories, the contract laboratory employed to clone the HAT-DHFR fusion polypeptide. The letter described in detail the procedure used for cloning the DHFR gene into the KpnI site of the pHS20 vector in order to generate the HAT-DHFR fusion polypeptide.
 - d. Page 4 of Exhibit A provides the N-terminal portion of the nucleic acid and amino acid sequence of the HAT-DHFR fusion protein encoded vector generated by ATG Laboratories. The amino acid sequence of the metal ion affinity tag is shown in the last two rows and is highlighted and shown in reverse order.
4. The disclosure appearing in Exhibit A clearly shows conception and reduction to practice of the claimed invention of the present application.
5. Accordingly, the evidence provided in Exhibit A establishes that the claimed invention of the above captioned application was conceived and reduced to practice prior to July 1998.
6. We do hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by

fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Respectfully submitted,

Date: 7/7/05

Grigoriy S. Tchaga
Grigoriy S. Tchaga

Date: 7/1/05

G. Jokhadze
George G. Jokhadze

Attachments: Exhibit A



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Exhibit A

1. Writing HAT manual

2. Purification of HAT-DHFR (20 mer) by FPLC under native and by batch/gravity flow under denaturing conditions.

A. FPLC

Load at 0 mM imidazole, wash at 5 mM imidazole and elution at 150 mM imidazole.

Sample: 300 mg of cells

Flow rate: 1 mL/min.

Fraction size: 1 mL

Quite good recovery and purity, similar to that of the 32 mer HAT-GFPuv.

B. Batch/gravity

Purification protocol - the same as for the FPLC.

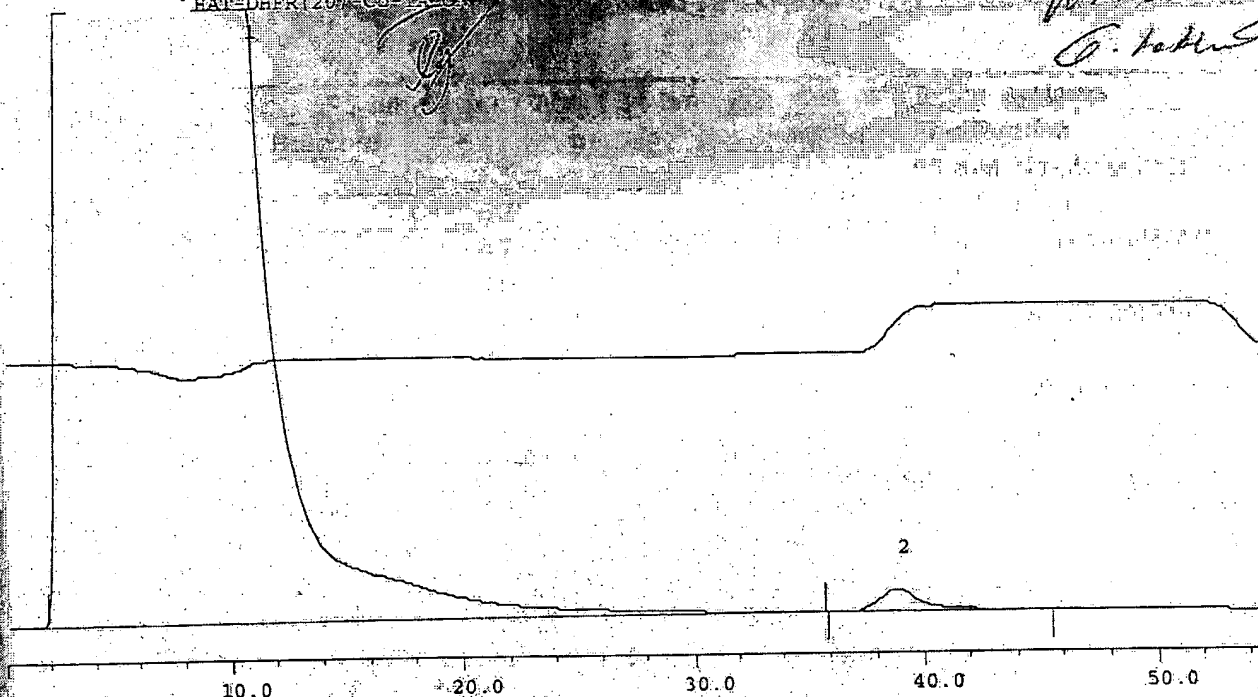
Sample: 100 mg of cells.

Obs! Binding at 6 M Guanidinium.HCl not quite strong as the one with the 32 mer - the HAT-DHFR is eluted with 5 mM imidazole (more than 70 %) but even there it is very pure.

The same purification should be repeated with wash at 0 mM imidazole and immediate elution at 150 mM imidazole.

HAT-DHFR (20)-Co-TALON

W. L. ...
C. ...

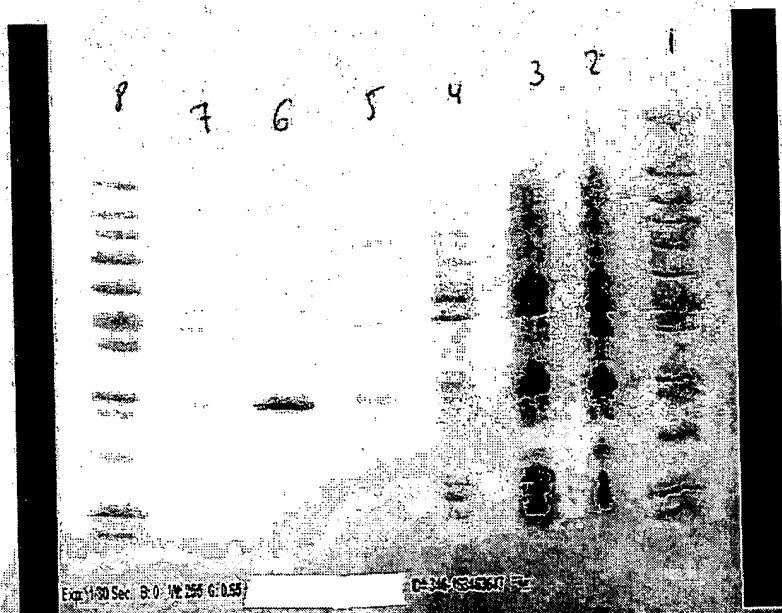


Sample: HAT-DHFR 20-mer - 2 mL
 Mon. sens. 2.0 AUFS 254 nm
 Column: Co(II)-TALON Superflow 6 3.5x1.0 cm.i.d.
 Rate: 1 mL/min
 A. 50 mM Na-phosphate; 0.3 M NaCl pH 7.0
 B. 20 mM Na-phosphate; 0.3 M NaCl; 150 mM imidazole pH 7.0
 Run: B=3.3% for 35 min; B=100% for 15 min

5.000000 Samples/Sec/Channel
 Manual Baseline and Peaks
 Abs. 254 nm -156.2 to 156.2 Millivolts

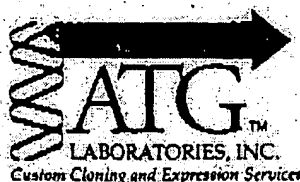
Peak	Time	Height	Area	%	Type
1	10.877	156.286	91314.749	99.47	BB++
2	38.943	3.581	482.053	0.53	BB

1. MW St.
2. SDS extr. cell Before ind.
3. OS
4. Non ads.
5. Wash x 10
6. HAT-DHFR x5
7. OS 110
8. MW St.



Exp 100 Sec 8.0 10 254 6.033

0-14 13467



TO: Paul Nelson, Ph.D.
Clontech Laboratories, Inc.

FROM: Laura T. Kakach

Final Report: Cloning DHFR gene into pHS20

The purpose of this project was to generate by PCR the DHFR gene from pQE40w and clone it into the KpnI site of pHS20. The strategy used is described.

The PCR primers were designed to amplify the coding region of the DHFR gene in pQE40w (without the ATG at the front end) to the Kpn I site present in the plasmid. All primers were purchased from Life Technologies, Inc. (Gaithersburg, MD). The front end primer, P57-S138 contains a KpnI site just upstream of the start of the DHFR gene to facilitate cloning. The Project Primer Catalog Report which is included with this report shows all the primers used for this project. In addition, the map of pQE40w is included and the primer locations are indicated on the map.

The PCR was carried out in duplicate using pQE40w as a template and the PCR primers P57-S138 and P57-A737. PCR products of the expected size (599 bp) were generated in both reactions. The PCR reactions were extracted with phenol, then chloroform, and ethanol precipitated. The DNA was then subjected to restriction digestion with KpnI and a fragment of 578 bp was gel purified. This fragment was cloned into the KpnI site of pHS20. Transformants were screened for the presence of a 578 bp KpnI fragment, as well as a 326 bp SacI fragment. One clone from each independent PCR reaction was chosen for sequence analysis. The clones were named pHSDHFR-A3 and pHSDHFR-B1. A map of pHS20 (named pUCHS20.MPD) is included.

Four sequencing reactions were carried out for each clone. Full sequence analysis was carried out for the A3 clone. The identification of the sequencing reactions is included with the report. The sequence of the DHFR gene in the A3 clone matched the sequence of the DHFR gene in the pQE40w plasmid with one exception. Nucleotide 606 on the map named PHSDHFR2 is an A, but both clones showed the base to be a G. This nucleotide change is silent in the DHFR gene, the affected codon is CTT to CTC, which both encode leucine. The sequence project data is included, named DHFRA3.SQD. The map of the plasmid is also included, named PHSDHFR2.MPD.

Finally, a photograph is included showing both A3 and B1 clones digested each with KpnI and SacI. The KpnI digests show a fragment approximately 575 bp as expected. The SacI digests show both clones to be in the orientation drawn in the map, which is in the same orientation as the lacZ coding region.

HSDHFR2.MPD (1 > 3335) Site and Sequence

Bst98 I

TCCTGGTAAACAGAACTGCCTCCGACTATCCAAACCATGTCTACTTTACTTGCCAATTCGGTTGTTCAATAAGTCTTAAGGCATCATCC
AGGACCATTGTCTTGACGGAGGCTGATAGGTTTGGTACAGATGAAATGAACGGTTAAGGCCAACAAGTTATTCAGAATTCGGTAGTAGG

720

Glu. Gln Tyr Val Ser Ser Gly Gly Val Ile Trp Val Met Asp Val Lys Ser Ala Leu Glu Pro Gln Glu Ile Leu Arg Leu Ala Asp Asp
dhfr

EcoICR I

Sac I

BssS I

BstX I

BseR I

Vsp I

Pra I

AAACTTTTGGCAAGAAAATGAGCTCCTCGTGGTGGTCTTTGAGTTCTCTACTGAGAACTATATTAATTCTGTCCTTTAAAGGTCGATTC
TTTGAAAACCGTTCTTTTACTCGAGGAGCACCACCAAGAACTCAAGAGATGACTCTTGATATAATTAAGACAGGAAATTTCCAGCTAAG

810

Leu Ser Lys Ala Leu Phe His Ala Gly Arg Pro Pro Glu Lys Leu Glu Arg Ser Leu Val Ile Asn Ile Arg Asp Lys Leu Pro Arg Asn
dhfr

SaxA I

AlwN I

Ear I

Eco57 I

TTCTCAGGAATGGAGAACCAGGTTTTCTACCCATAATCACCAGATTCTGTTTACCTTECACTGAAGAGGTTGTGGTCATTCTTTGGAAG
AAGAGTCCTTACCTCTTGGTCCAAAAGGATGGGTATTAGTGGTCTAAGACAAATGGAAGGTGACTTCTCCAACACCAGTAAGAAACCTTC

900

Lys Glu Pro Ile Ser Phe Trp Thr Lys Arg Gly Met Ile Val Leu Asn Gln Lys Gly Glu Val Ser Ser Thr Thr Thr Met Arg Gln Phe
dhfr

Sca I

Xmn I

BsrB I

Bsa I

Van91 I

Pvu I

TACTTGAACCTCGTTCTCTGAGCGGAGGCCAGGGTAGGTCTCCGTTCTTGCCAATCCCCATATTTTGGGACACGGCGACGATCGAGTTCAAT
ATGAACTTGAGCAAGGACTCGCTCCGGTCCCATCCAGAGGCAAGAACGGTTAGGGGTATAAAACCTGTGCCGCTGCTAGCTCAAGTTA

99C

Tyr Lys Phe Glu Asn Arg Leu Pro Pro Trp Pro Leu Asp Gly Asn Lys Gly Ile Gly Met Asn Gln Ser Val Ala Val Ile Ser Asn Leu
dhfr

Acc85 I

Kpn I

Xma I

Sma I

BamH I

Sal I

Tth111 I

Cla I

BseR I

GGTCGAACGGTACCCGGGGATCCGTCGACTTTGTCATCGTCATCGATCTTGTGTGGGCATGAGCGTGCTCCTCTTTGTGGACATTGTGG
CCAGETTGCCATGGGCCCTAGGCAGCTGAAACAGTAGCAGTAGCTAGAACAAACACCCGTAETGCGACGAGGAGAAACACCTGTAACACC

108

Pro Arg Val Thr Gly Pro Ser Gly Asp Val Lys Asp Asp Asp Asp Ile Lys Asn His Ala His Ala His Glu Glu Lys His Val Asn His
dhfr

Hind III

BsrB I

ATGAGATGATCCTTCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCTGTGTGAAATTGTTATCCGCTCACAATTCACACAACATACG
TACTCTACTAGGAAGTTTGAACCGCATTAGTACCAGTATCGACAAAGGACACACTTAACAATAGGCGAGTGTTAAGGTGTGTTGTATGC

11

Ile Leu His Asp Lys Leu Ser Pro Thr Ile Met

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